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Review Article

Mucosal anti-caries DNA vaccine: a new approach to induce protective immunity against streptococcus mutans

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Abstract: Dental caries is caused by specific types of acid-producing bacteria such as Streptococcus mutans when the pH at the tooth surface drops below 5.5. Despite progress on the development of anti-caries therapies, the morbidity of dental caries keeps increasing worldwide. Anti-caries DNA vaccine offers a promising strategy to induce specific and protective immunity to eliminate cariogenic bacteria. Here we propose the development of an innovative dental vaccine for prophylactic immunization against S. mutans, especially its major virulence factors Glucosyltransferases (GTFs) and surface protein PAc. Meanwhile, adjuvants effectively improve the immunogenicity of anti-caries DNA vaccines and offer protective long-term immunity. In addition, understanding of the signals that regulate the colonization and growth of S. mutans in dental biofilms may help develop refined and advanced techniques to get rid of cariogenic bacteria.

Keywords: Dental caries, anti-caries DNA vaccine, nasal mucosa, streptococcus mutans

Introduction

Dental caries is the most common bacterial infectious disease in human and has become the primary cause of oral pain and tooth loss, seriously affecting the quality of life of the patients [1]. Individuals are susceptible to dental caries all the lifetime [2]. Dental caries is caused by specific types of acid-producing bacteria (e.g., Streptococcus mutans) when the pH at the tooth surface drops below 5.5 [3]. Currently, the main methods to prevent caries disease are adding fluoride in public water, fluoride toothpaste, mouthwash with antimicrobial drug, and the use of xylitol [4]. However, children identified with intellectual disability living in rural areas are highly susceptibile to infection because of immune status and the lack of availability and flexibility of dental appointments. Prevention or control of dental caries can not be achieved by reliance only on current methods and models of dental care, especially for mentally disabled children living in the countryside [5]. Therefore, it is urgent to develop new and efficient methods for caries control and prevention. The design of anti-caries DNA vaccine offers a promising strategy to induce specific and protective immunity to eliminate cariogenic bacteria. Anti-caries DNA vaccines have shown the ability to inhibit S. mutans infection and protect host against dental caries [6, 7].

Dental caries and streptococcus mutans

Dental caries results from the interactions between bacteria that produce acid, a substrate that the bacteria can metabolise, and many host factors that include teeth and saliva. Endogenous bacteria (largely S. mutans and Lactobacillus spp) in the biofilm produce weak organic acids as a by-product of metabolism of fermentable carbohydrates [8-10]. The acid causes local pH value to fall below a critical value resulting in the demineralisation of tooth tissues [10]. If the diffusion of calcium, phosphate or carbonate out of the tooth is allowed to continue, cavitation will eventually develop. Demineralisation can be reversed in the early stages through the uptake of calcium, phos-

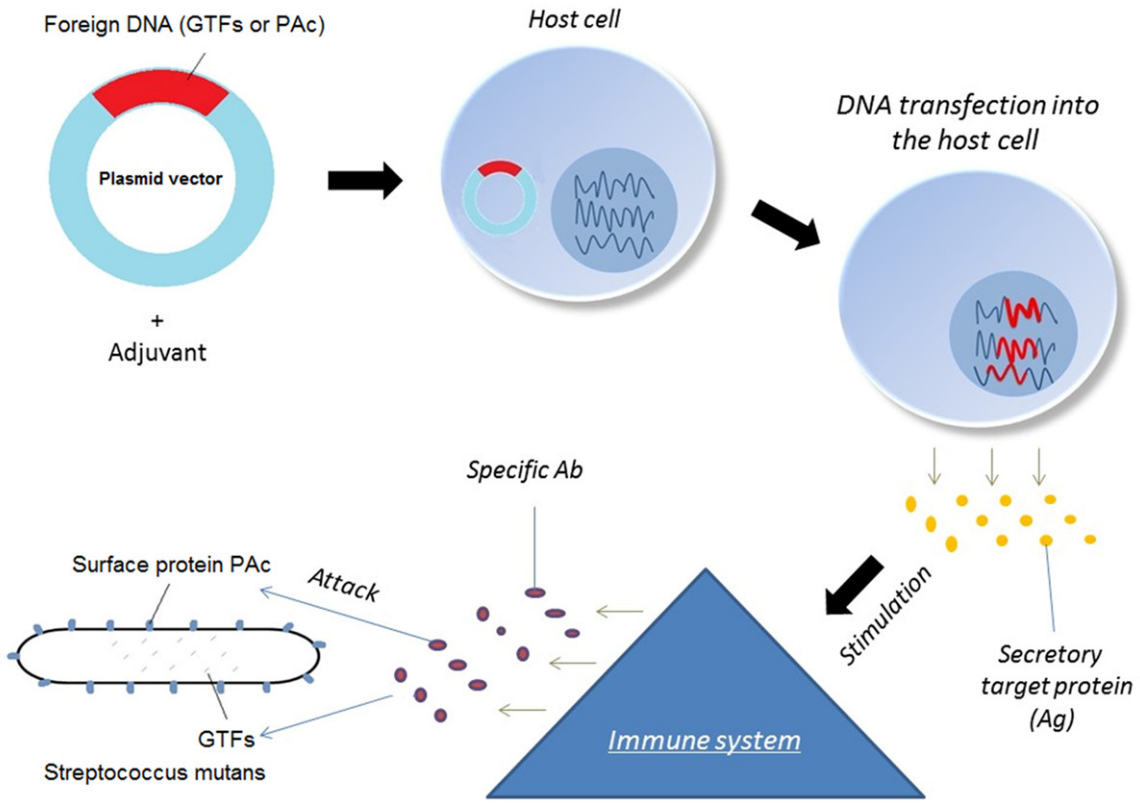

Figure 1. Host cell transfection by plasmid vector with foreign DNA and the induction of protective immunity against GTFs and PAc, two major virulence factors of S. mutans.

phate and fluoride. Whether dental caries progresses, stops, or reverses is dependent on a balance between the demineralisation and the remineralisation.

S. mutans has been strongly implicated as the principal pathogen of dental caries in human [11, 12]. There are two major virulence factors for S. mutans infection: Glucosyltransferases (GTFs) and a surface protein PAc (such as antigen I/II, B and P1) [13, 14]. GTFs function as enzymes to catalyze water-soluble and water-insoluble glucan synthesis from sucrose. The sucrose-dependent attachment of S. mutans to the acquired pellicles on tooth surfaces is mediated through its N-terminal catalytic (CAT) region and the C-terminal glucan-binding (GLU) region [15]. GTFs are involved in many processes during extracellular polysaccharides biosynthesis of which S. mutans can make use to produce organic acids that demineralize the tooth enamel and cause dental caries [16, 17]. PAc mediates the initial attachment of S. mutans to tooth surfaces via its N-terminal alanine-rich region (A region) and middle proline-rich region (P region) [18]. The adhesin known as PAc of cariogenic pathogen S. mutans is a target of protective immunity and candidate vaccine antigen [19, 20]. Antibodies could block the receptors necessary for colonization (e.g., surface proteins PAc or adhesins) or accumulation (e.g., GTFs), and inactivate GTF enzymes responsible for glucan formation [21]. Directing antigens to antigen presenting cells (APCs) has been demonstrated to be an efficient strategy to enhance immune response induced by DNA vaccination [22].

Anti-caries DNA vaccination

Colonisation by S. mutans and other cariogenic bacteria at a young age could be a key risk factor for caries development [23]. Anti-caries DNA vaccination is a new immunization strategy against dental infectious disease, and has many advantages over traditional vaccines, such as easy preparation and administeration, and long-lasting cellular and humoral immune responses [24]. Anti-caries DNA vaccination may function as a multi-epitope vaccine and

stimulate strong humoral and cell-mediated immune response targeting S. mutans associated antigens. However, anti-caries DNA vaccine has low immunogenicity because of the low efficiency of uptake [22].

Intranasal installation of antigen, which targets the nasal-associated lymphoid tissue (NALT) [25], has been used to induce immunity to many bacterial antigens, including those associated with streptococcal colonization and accumulation. Intranasal route administration of S. mutans antigens or functional domains such as AgI/II, glucan-binding domain of GTF-B and flagellin in combination with mucosal adjuvants could induce protective immunity in rats after infection with cariogenic streptococci [26-29]. C-C chemokine ligand-19 (CCL-19) serves as an effective adjuvant for anti-caries DNA vaccine by inducing chemotactic migration of DCs to secondary lymphoid tissues, which leading to significantly increased level of serum PAc-specific IgG [29]. As a potent immune activator, flagellin is the ligand of the Toll-like receptor 5 (TLR5), which is one of the pathogen-associated molecular pattern (PAMP) receptors [30]. Recombinant flagellin protein derived from Salmonella (FliC) as a mucosal adjuvant for anti-caries DNA vaccine (pGJA-P/VAX) promoted the production of PAc-specific IgG in serum and salivary secretory IgA (S-IgA) in saliva of rats after intranasal immunization.

The whole DNA transfection process is briefly shown in **Figure 1**. Enhanced PAc-specific IgA responses in saliva were associated with the inhibition of S. mutans colonization of tooth surfaces and better protection with significant fewer caries lesions [28]. In addition, recombinant protein (KF-rPAc) composed of the flagellin derived from E. coli (KF) and target antigen PAc containing the A-P fragment of PAc from S. mutans (rPAc) induce rPAc-specific mucosal and systemic responses and provide efficient protection against caries following intranasal immunization [26]. Thus the design of suitable adjuvants such as FliC and KF-rPAc can effectively improve the immunogenicity of anti-caries DNA vaccines and offer protective long-term immunity.

Perspectives

Dental caries is a disease that needs to be managed all over a person's life. Anti-caries DNA vaccine makes caries-free life possible. The ideal anti-caries DNA vaccine should include the following properties: low cost, efficacy, safety, persistence, broadest coverage, work for both low- and high-risk populations, work with other immunizations, and easy administration by various routes. To increase the immunogenicity of anti-caries DNA vaccine, we proposed the design of a new-type targeted and adjuvanted anti-caries vaccine, which is composed of plasmid vector, target gene (GTFs & PAc) and adjuvant.

An aggressive tooth decay that affects the primary teeth of infants and toddlers can arise in early childhood [31]. In dental caries management, dental caries vaccines would be the first non-living vaccine to be applied by mucosal route during the first three years of life. Anti-caries DNA vaccine can induce S-IgA antibodies against S. mutans adherence and biofilms formation, thereby reducing the incidence of dental caries [32]. A mucosal delivery route can increase the immunogenicity of anti-caries DNA vaccines [27]. An intranasal or oral application of GTFs or PAc-based vaccine may be the best, provided the children are otherwise healthy and parents have the ability to make the office visits as immunizations require repeated vaccinations. Additionally, the frequency, time-interval and doses of anti-caries DNA vaccines must be strictly controlled in order to avoid immune-tolerance. On the other hand, the potential side effects of anti-caries vaccines should be addressed. For example, some polypeptides on the cell membranes of S. mutans immunologically cross-reacted with human heart tissue and skeletal muscle protein myosin [33, 34]. Therefore, it is important to identify antigens with potential cross-reactivity and exclude them in the design of anti-caries vaccines. Further experiments with animal studies, vaccine standardization, adjuvant co-stimulatory methods, potential toxicity including autoimmune responses, and the measurement of antibody and T-cell responses are needed to confirm beneficial outcomes of anti-caries immunotherapy.

In conclusion, an innovative dental vaccine for prophylactic immunization against S. mutans, especially its major virulence factors GTFs and PAc, will bring new hope for the management of dental caries. In addition, understanding of the

signals that regulate the colonization and growth of S. mutans in dental biofilms may help develop refined and advanced techniques to get rid of cariogenic bacteria.

Disclosure of conflict of interest

None.

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